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(54) Title: IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC GLYCOSYLTRANSFERASE					
(57) Abstract <p>The invention relates to nucleic acids which encode glycosyltransferase and are useful in producing cells and organs from one species which may be used for transplantation into a recipient of another species. It also relates to the production of nucleic acids which, when present in cells of a transplanted organ, result in reduced levels of antibody recognition of the transplanted organ.</p>					
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IMPROVED NUCLEIC ACIDS ENCODING A CHIMERIC
GLYCOSYLYLTRANSFERASE

Field of the Invention

5 The present invention relates to nucleic acids which encode glycosyltransferase and are useful in producing cells and organs from one species which may be used for transplantation into a recipient of another species. Specifically the invention concerns production of
10 nucleic acids which, when present in cells of a transplanted organ, result in reduced levels of antibody recognition of the transplanted organ.

Background of the Invention

15 The transplantation of organs is now practicable, due to major advances in surgical and other techniques. However, availability of suitable human organs for transplantation is a significant problem. Demand outstrips supply. This has caused researchers to investigate the
20 possibility of using non-human organs for transplantation.

25 Xenotransplantation is the transplantation of organs from one species to a recipient of a different species. Rejection of the transplant in such cases is a particular problem, especially where the donor species is more distantly related, such as donor organs from pigs and sheep to human recipients. Vascular organs present a
30 special difficulty because of hyperacute rejection (HAR).

35 HAR occurs when the complement cascade in the recipient is initiated by binding of antibodies to donor endothelial cells.

40 Previous attempts to prevent HAR have focused on two strategies : modifying the immune system of the host by inhibition of systemic complement formation (1,2), and antibody depletion (3,4). Both strategies have been shown
45 to prolong xenograft survival temporarily. However, these methodologies are therapeutically unattractive in that they are clinically impractical, and would require chronic

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immunosuppressive treatments. Therefore, recent efforts to inhibit HAR have focused on genetically modifying the donor xenograft. One such strategy has been to achieve high-level expression of species-restricted human complement 5 inhibitory proteins in vascularized pig organs via transgenic engineering (5-7). This strategy has proven to be useful in that it has resulted in the prolonged survival of porcine tissues following antibody and serum challenge (5,6). Although increased survival of the transgenic 10 tissues was observed, long-term graft survival was not achieved (6). As observed in these experiments and also with systemic complement depletion, organ failure appears to be related to an acute antibody-dependent vasculitis (1,5).

15 In addition to strategies aimed at blocking complement activation on the vascular endothelial cell surface of the xenograft, recent attention has focused on identification of the predominant xenogeneic epitope recognised by high-titre human natural antibodies. It is 20 now accepted that the terminal galactosyl residue, Gal- α (1,3)-Gal, is the dominant xenogeneic epitope (8-15). This epitope is absent in Old World primates and humans because the α (1,3)-galactosyltransferase (gal-transferase or GT) is non-functional in these species. DNA sequence comparison of 25 the human gene to α (1,3)-galactosyltransferase genes from the mouse (16,17), ox (18), and pig (12) revealed that the human gene contained two frameshift mutations, resulting in a non-functional pseudogene (20,21). Consequently, humans and Old World primates have pre-existing high-titre 30 antibodies directed at this Gal- α (1,3)-Gal moiety as the dominant xenogeneic epitope.

One strategy developed was effective to stably reduce the expression of the predominant Gal- α (1,3)-Gal epitope. This strategy took advantage of an intracellular 35 competition between the gal-transferase and α (1,2)-fucosyltransferase (H-transferase) for a common acceptor substrate. The gal-transferase catalyses the transfer of a

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terminal galactose moiety to an N-acetyl lactosamine acceptor substrate, resulting in the formation of the terminal Gal- α (1,3)-Gal epitope. Conversely, H-transferase catalyses the transfer of a fucosyl residue to the N-acetyl lactosamine acceptor substrate, and generates a fucosylated N-acetyl lactosamine (H-antigen, i.e., the O blood group antigen), a glycosidic structure that is universally tolerated. Although it was reported that expression of human H-transferase transfected cells resulted in high level expression of the non-antigenic H-epitope and significantly reduced the expression of the Gal- α (1,3)-Gal xenoepitope, there are still significant levels of Gal- α (1,3)-Gal epitope present on such cells.

15 Summary of the Invention

In view of the foregoing, it is an object of the present invention to further reduce levels of undesirable epitopes in cells, tissues and organs which may be used in transplantation.

20 In work leading up to the invention the inventors surprisingly discovered that the activity of H transferase may be further increased by making a nucleic acid which encodes a H transferase catalytic domain but is anchored in the cell at a location where it is better able to compete
25 for substrate with gal transferase. Although work by the inventors focused on a chimeric H transferase, other glycosyltransferase enzymes may also be produced in accordance with the invention.

Accordingly, in a first aspect the invention
30 provides a nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby when said nucleic acid is expressed in a cell said chimeric enzyme is located in
35 an area of the cell where it is able to compete for substrate with a second glycosyltransferase, resulting in reduced levels of a product from said second

glycosyltransferase.

Preferably the nucleic acid is in an isolated form; that is the nucleic acid is at least partly purified from other nucleic acids or proteins.

5 Preferably the nucleic acid comprises the correct sequences for expression, more preferably for expression in a eukaryotic cell. The nucleic acid may be present on any suitable eukaryotic expression vector such as pcDNA (Invitrogen). The nucleic acid may also be present on other 10 vehicles whether suitable for eukaryotes or not, such as plasmids, phages and the like.

15 Preferably the catalytic domain of the first glycosyltransferase is derived from H transferase, secretor sialyltransferase, a galactosyl sulphating enzyme or a phosphorylating enzyme.

20 The nucleic acid sequence encoding the catalytic domain may be derived from, or similar to a glycosyltransferase from any species. Preferably said species is a mammalian species such as human or other 25 primate species, including Old World monkeys, or other mammals such as ungulates (for example pigs, sheep, goats, cows, horses, deer, camels) or dogs, mice, rats and rabbits. The term "similar to" means that the nucleic acid is at least partly homologous to the glycosyltransferase genes described above. The term also extends to fragments 30 of and mutants, variants and derivatives of the catalytic domain whether naturally occurring or man made.

35 Preferably the localisation signal is derived from a glycosyltransferase which produces glycosylation patterns which are recognised as foreign by a transplant recipient. More preferably the localisation signal is derived from $\alpha(1,3)$ galactosyltransferase. The effect of this is to downregulate the level of Gal- $\alpha(1,3)$ -Gal produced in a cell when the nucleic acid is expressed by the cell.

The nucleic acid sequence encoding the localisation signal may be derived from any species such as

those described above. Preferably it is derived from the same species as the cell which the nucleic acid is intended to transform i.e., if pig cells are to be transformed, preferably the localization signal is derived from pig.

5 More preferably the nucleic acid comprises a nucleic acid sequence encoding the catalytic domain of H transferase and a nucleic acid sequence encoding a localisation signal from Gal transferase. Still more preferably both nucleic acid sequences are derived from 10 pigs. Even more preferably the nucleic acid encodes gtHT described herein.

15 The term "nucleic acid" refers to any nucleic acid comprising natural or synthetic purines and pyrimidines. The nucleic acid may be DNA or RNA, single or double stranded or covalently closed circular.

20 The term "catalytic domain" of the chimeric enzyme refers to the amino acid sequences necessary for the enzyme to function catalytically. This comprises one or more contiguous or non-contiguous amino acid sequences. Other non-catalytically active portions also may be included in the chimeric enzyme.

25 The term "glycosyltransferase" refers to a polypeptide with an ability to move carbohydrates from one molecule to another.

30 The term "derived from" means that the catalytic domain is based on, or is similar, to that of a native enzyme. The nucleic acid sequence encoding the catalytic domain is not necessarily directly derived from the native gene. The nucleic acid sequence may be made by polymerase chain reaction (PCR), constructed *de novo* or cloned.

35 The term "localisation signal" refers to the amino acid sequence of a glycosyltransferase which is responsible for anchoring it in location within the cell. Generally localisation signals comprise amino terminal "tails" of the enzyme. The localisation signals are derived from a second glycosyltransferase, the activity of which it is desired to minimise. The localisation of a

5 catalytic domain of a first enzyme in the same area as the second glycosyltransferase means that the substrate reaching that area is likely to be acted on by the catalytic domain of the first enzyme, enabling the amount of substrate catalysed by the second enzyme to be reduced.

The term "area of the cell" refers to a region, compartment or organelle of the cell. Preferably the area of the cell is a secretory organelle such as the Golgi apparatus.

10 In another aspect the invention provides an isolated nucleic acid molecule encoding a localisation signal of a glycosyltransferase. Preferably the signal encoded comprises an amino terminus of said molecule; more preferably it is the amino terminus of gal transferase. The 15 gal transferase may be derived from or based on a gal transferase from any mammalian species, such as those described above. Particularly preferred sequences are those derived from pig, mouse or cattle.

20 In another aspect the invention relates to a method of producing a nucleic acid encoding a chimeric enzyme, said enzyme comprising a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase whereby when said nucleic acid is expressed in a cell said chimeric enzyme is located in 25 an area of the cell where it is able to compete for substrate with a second glycosyltransferase said method comprising operably linking a nucleic acid sequence encoding a catalytic domain from a first glycosyltransferase to a nucleic acid sequence encoding a 30 localisation signal of a second glycosyltransferase.

The term "operably linking" means that the nucleic acid sequences are ligated such that a functional protein is able to be transcribed and translated.

35 Those skilled in the art will be aware of various techniques for producing the nucleic acid. Standard techniques such as those described in Sambrook et al may be employed.

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Preferably the nucleic acid sequences are the preferred sequences described above.

In another aspect the invention provides a method of reducing the level of a carbohydrate exhibited on the 5 surface of a cell, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said 10 chimeric enzyme is located in an area of the cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

15 The term "reducing the level of a carbohydrate" refers to lowering, minimising, or in some cases, ablating the amount of carbohydrate displayed on the surface of the cell. Preferably said carbohydrate is capable of stimulating recognition of the cell as "non-self" by the 20 immune system of an animal. The reduction of such a carbohydrate therefore renders the cell, or an organ composed of said cells, more acceptable to the immune system of a recipient animal in a transplant situation or gene therapy situation.

25 The term "causing a nucleic acid to be expressed" means that the nucleic acid is introduced into the cell (i.e. by transformation/transfection or other suitable means) and contains appropriate signals to allow expression in the cells.

30 The cell may be any suitable cell, preferably mammalian, such as that of a New World monkey, ungulate (pig, sheep, goat, cow, horse, deer, camel, etc.) or other species such as dogs.

35 In another aspect the invention provides a method of producing a cell from one species (the donor) which is immunologically acceptable to another species (the recipient) by reducing levels of carbohydrate on said cell

which cause it to be recognised as non-self by the other species, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a 5 first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of 10 producing said carbohydrate.

The term "immunologically acceptable" refers to producing a cell, or an organ made up of numbers of the cell, which does not cause the same degree of immunological reaction in the recipient species as a native cell from the 15 donor species. Thus the cell may cause a lessened immunological reaction, only requiring low levels of immunosuppressive therapy to maintain such a transplanted organ or no immunosuppression therapy.

The cell may be from any of the species mentioned 20 above. Preferably the cell is from a New World primate or a pig. More preferably the cell is from a pig.

The invention extends to cells produced by the above method and also to organs comprising the cells.

The invention further extends to non-human 25 transgenic animals harbouring the nucleic acid of the invention. Preferably the species is a human, ape or Old World monkey.

The invention also extends to the proteins produced by the nucleic acid. Preferably the proteins are 30 in an isolated form.

In another aspect the invention provides an expression unit which expresses the nucleic acid of the invention, resulting in a cell which is immunologically acceptable to an animal having reduced levels of a 35 carbohydrate on its surface, which carbohydrate is recognised as non-self by said species. In a preferred embodiment, the expression unit is a retroviral packaging

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cell, cassette, a retroviral construct or retroviral producer cell.

Preferably the species is a human, ape or Old World monkey.

5 The retroviral packaging cells or retroviral producer cells may be cells of any animal origin where it is desired to reduce the level of carbohydrates on its surface to make it more immunologically acceptable to a host. Such cells may be derived from mammals such as 10 canine, rodent or ruminant species and the like.

The retroviral packaging and/or producer cells may be used in applications such as gene therapy. General methods involving use of such cells are described in PCT/US95/07554 and the references discussed therein.

15 The invention also extends to a method of producing a retroviral packaging cell or a retroviral producer cell having reduced levels of a carbohydrate on its surface wherein the carbohydrate is recognised as non-self by a species, comprising transforming/transfected a 20 retroviral packaging cell or a retroviral producer cell with the nucleic acid of the invention under conditions such that the chimeric enzyme is produced.

Brief Description of the Drawings

25

Figure 1 Schematic diagram of normal and chimeric glycosyltransferases

The diagram shows normal glycosyltransferases porcine $\alpha(1,3)$ galactosyltransferase (GT) and human $\alpha(1,2)$ fucosyltransferase (HT), and chimeric transferases ht-GT in which the cytoplasmic domain of GT has been 30 completely replaced by the cytoplasmic domain of HT, and gt-HT in which the cytoplasmic domain of HT has been 35 entirely replaced by the cytoplasmic domain of GT. The protein domains depicted are cytoplasmic domain CYTO, transmembrane domain TM, stem region STEM, catalytic domain CATALYTIC. The numbers refer to the amino acid sequence of

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the corresponding normal transferase.

Figure 2 Cell surface staining of COS cells transfected with normal and chimeric transferases

5 Cells were transfected with normal GT or HT or with chimeric transferases gt-HT or ht-GT and 48h later were stained with FITC-labelled lectin IB4 or UEA1. Positive-staining cells were visualised and counted by fluorescence microscopy. Results are from at least three 10 replicates and values are +/- SEM.

Figure 3. RNA analysis of transfected COS cells

15 Northern blots were performed on total RNA prepared from COS cells transfected: Mock, mock- transfected; GT, transfected with wild-type GT; GT1-6/HT, transfected with chimeric transferase gt-HT; GT1-6/HT + 20 HT1-8/GT, co-transfected with both chimeric transferases gt-HT and ht-GT; HT1-8/GT, transfected with chimeric transferase ht-GT; HT, transfected with normal HT; GT + HT, co-transfected with both normal transferases GT and HT. Blots were probed with a cDNA encoding GT (Top panel), HT (Middle panel) or g-actin (Bottom panel).

Figure 4. Enzyme kinetics of normal and chimeric 25 glycosyltransferases

Lineweaver-Burk plots for $\alpha(1,3)$ galactosyltransferase (\square) and $\alpha(1,2)$ fucosyltransferase (\blacksquare) to determine the apparent K_m values for N-acetyl lactosamine. Experiments were performed in triplicate, 30 plots shown are of mean values of enzyme activity of wild-type transferases, GT and HT, and chimeric proteins ht-GT and gt-HT in transfected COS cell extracts using phenyl-B-D Gal and N-acetyl lactosamine as acceptor substrates.

35 Figure 5. Staining of cells co-transfected with chimeric transferases

Cells were co-transfected with cDNAs encoding

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normal transferases GT + HT (panels A, B), with chimeric transferases gt-HT + ht-GT (panels C, D), with HT + ht-GT (panels E, F) or with GT + gt-HT (panels G, H) and 48h later were stained with FITC-labelled lectin IB4 (panels A, 5 C, E, G) or UEAI (panels B, D, F, H).

Figure 6 is a representation of the nucleic acid sequence and corresponding amino acid sequence of pig secretor.

10 Figure 7 is a representation of the nucleic acid sequence and corresponding amino acid sequence of pig H.

Figure 8 Cell surface staining of pig endothelial cell line (PIEC) transfected with chimeric $\alpha(1,2)$ -fucosyltransferase. Cells were transfected and clones exhibiting stable integration were stained with UEAI lectin and visualised by fluorescence microscopy.

20 Figure 9 Screening of chimeric $\alpha(1,2)$ -fucosyltransferase transferase in mice. Mice were injected with chimeric $\alpha(1,2)$ -fucosyltransferase and the presence of the transferase was analysed by dot blots.

Description of the Preferred Embodiment

25 The nucleic acid sequences encoding the catalytic domain of a glycosyltransferase may be any nucleic acid sequence such as those described in PCT/US95/07554, which is herein incorporated by reference, provided that it encodes a functional catalytic domain with the desired 30 glycosyltransferase activity.

Preferred catalytic domains from glycosyltransferase include H transferase and secretor. Preferably these are based on human or porcine sequences.

35 The nucleic acid sequences encoding the localisation signal of a second transglycosylase may be any nucleic acid sequence encoding a signal sequence such as signal sequences disclosed in P A Gleeson, R D Teasdale &

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J Bourke, Targeting of proteins to the Golgi apparatus. Glycoconjugate J. (1994) 11: 381-394. Preferably the localisation signal is specific for the Golgi apparatus, more preferably for that of the trans Golgi. Still more 5 preferably the localisation signal is based on that of Gal transferase. Even more preferably the localisation signal is based on porcine, murine or bovine sequences. Even more preferably the nucleic acid encodes a signal sequence with following amino acid sequence (in single letter code):
10 MNVKGR (porcine), MNVKGK (mouse) or MVVKGK (bovine).

Vectors for expression of the chimeric enzyme may be any suitable vector, including those disclosed in PCT/US95/07554.

15 The nucleic acid of the invention can be used to produce cells and organs with the desired glycosylation pattern by standard techniques, such as those disclosed in PCT/US95/07554. For example, embryos may be transfected by standard techniques such as microinjection of the nucleic acid in a linear form into the embryo (22). The embryos are 20 then used to produce live animals, the organs of which may be subsequently used as donor organs for implantation.

25 Cells, tissues and organs suitable for use in the invention will generally be mammalian cells. Examples of suitable cells and tissues such as endothelial cells, hepatic cells, pancreatic cells and the like are provided in PCT/US95/07554.

The invention will now be described with reference to the following non-limiting Examples.

ABBREVIATIONS

The abbreviations used are bp, base pair(s); FITC, fluorescein isothiocyanate; GT, 5 galactosyltransferase; H substance, α (1,2)fucosyl lactosamine; HT, α (1,2)fucosyltransferase; PCR, polymerase chain reaction; Example 1 Cytoplasmic domains of glycosyltransferases play a central role in the temporal action of enzymes

10

EXPERIMENTAL PROCEDURES

Plasmids - The plasmids used were prepared using standard techniques (7); pGT encodes the cDNA for the porcine α (1,3)galactosyltransferase (23), pHT encodes the 15 cDNA for the α (1,2)fucosyltransferase (human) (25).

Chimeric glycosyltransferase cDNAs were generated by polymerase chain reaction as follows: an 1105 bp product ht-GT was generated using primers corresponding to the 5' end of ht-GT (5'-GCGGATCCATGTGGCTCCGGAGCC

20 ATCGTCAGGTGGTTCTGTCAATGC TGCTTG-3') coding for nucleotides 1-24 of HT (25) followed immediately by nucleotides 68-89 of GT (8) and containing a BamH1 site (underlined) and a primer corresponding to the 3' end of ht-GT (5'-
GCTCTAGAGCGTCAGATGTTATT TCTAACCAAATTATAC-3') containing 25 complementarity to nucleotides 1102-1127 of GT with an XbaI site downstream of the translational stop site (underlined); an 1110 bp product gt-HT was generated using primers corresponding to the 5' end of gt-HT (5'-
GCGGATCCCATGAATGTCAAAGGAAGACTCTGCCTGGCCT TCCTGC-3') coding 30 for nucleotides 49-67 of GT followed immediately by nucleotides 25-43 of HT and containing a BamH1 site (underlined) and a primer corresponding to the 3' end of gt-HT (5'-GCTCTAGAGCCTCAAGGCTTAG CCAATGTCCAGAG-3') containing complementarity to nucleotides 1075-1099 of HT 35 with a XbaI site downstream of the translational stop site (underlined). PCR products were restricted BamH1/XbaI, gel-purified and ligated into a BamH1/XbaI digested pcDNA1

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expression vector (Invitrogen) and resulted in two plasmids pht-GT (encoding the chimeric glycosyltransferase ht-GT) and pgt-HT (encoding the chimeric glycosyltransferase gt-HT) which were characterised by restriction mapping,

5 Southern blotting and DNA sequencing .

Transfection and Serology - COS cells were maintained in Dulbecco's modified Eagles Medium (DMEM) (Trace Biosciences Pty. Ltd. , Castle Hill, NSW, Australia) and were transfected (1-10 μ g DNA/5 x 10⁵ cells)

10 using DEAE-Dextran (26); 48h later cells were examined for cell surface expression of H substance or Gal- α (1,3)-Gal using FITC-conjugated lectins: IB4 lectin isolated from Griffonia simplicifolia (Sigma, St. Louis, MO) detects Gal- α (1,3)-Gal (27); UEAI lectin isolated from Ulex

15 europaeus (Sigma, St. Louis, MO) detects H substance (28).

H substance was also detected by indirect

immunofluorescence using a monoclonal antibody (mAb) specific for the H substance (ASH-1952) developed at the Austin Research Institute, using FITC-conjugated goat anti-

20 mouse IgG (Zymed Laboratories, San Francisco, CA) to detect mAb binding. Fluorescence was detected by microscopy.

RNA Analyses - Cytoplasmic RNA was prepared from transfected COS cells using RNAzol (Bioteck Laboratories, Houston, TX), and total RNA was electrophoresed in a 1%

25 agarose gel containing formaldehyde, the gel blotted onto a nylon membrane and probed with random primed GT or HT cDNA.

Glycosyltransferase assays - Forty-eight hours after transfection, cells were washed twice with phosphate buffered saline and lysed in 1% Triton X-100/ 100 mM cacodylate pH 6. 5/ 25 mM MnCl₂, at 4°C for 30 min; lysates were centrifuged and the supernatant collected and stored at -70°C. Protein concentration was determined by the Bradford method using bovine serum albumin as standard

30 (29). Assays for HT activity (30) were performed in 25 μ l containing 3mM [GDP^{-14}C]fucose (specific activity 287 mCi/mmol, Amersham International), 5mM ATP, 50mM MOPS pH 6. 5, 20 mM MnCl₂, using 2-10 μ l of cell extract

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(approximately 15-20 μ g of protein) and a range of concentrations (7. 5 -75 mM) of the acceptor phenyl-B-D-galactoside (Sigma). Samples were incubated for 2h at 37°C and reactions terminated by the addition of ethanol and water. The amount of 14 C-fucose incorporated was counted after separation from unincorporated label using Sep-Pak C18 cartridges (Waters-Millipore, Millford, MA). GT assays (31) were performed in a volume of 25 μ l using 3mM UDP[3 H]-Gal (specific activity 189mCi/mmol, Amersham International), 5mM ATP, 100mM cacodylate pH 6. 5, 20mM MnCl₂ and various concentrations (1 -10 mM) of the acceptor N-acetyl lactosamine (Sigma). Samples were incubated for 2h at 37°C and the reactions terminated by the addition of ethanol and water. 3 H-Gal incorporation was counted after separation from non-incorporated UDP[3 H]-Gal using Dowex I anion exchange columns (BDH Ltd. , Poole, UK) or Sep-Pak Accell plus QMA anion exchange cartridges (Waters-Millipore, Millford, MA). All assays were performed in duplicate and additional reactions were performed in the absence of added acceptor molecules, to allow for the calculation of specific incorporation of radioactivity.

RESULTS

Expression of chimeric $\alpha(1,3)$ galactosyltransferase and $\alpha(1,2)$ fucosyltransferase cDNAs

We had previously shown that when cDNAs encoding $\alpha(1,3)$ galactosyltransferase (GT) and $\alpha(1,2)$ fucosyltransferase (HT) were transfected separately they could both function efficiently leading to expression of the appropriate carbohydrates: Gal- $\alpha(1,3)$ -Gal for GT and H substance for HT (32). However when the cDNAs for GT and HT were transfected together, the HT appeared to "dominate" over the GT in that H substance expression was normal, but Gal- $\alpha(1,3)$ -Gal was reduced. We excluded trivial reasons for this effect and considered that the localisation of the enzymes may be the reason. Thus, if the HT localisation signal placed the enzyme in an earlier temporal compartment

than GT, it would have "first use" of the N-acetyl lactosamine substrate. However, such a "first use" if it occurred, was not sufficient to adequately reduce GT. Two chimeric glycosyltransferases were constructed using PCR 5 wherein the cytoplasmic tails of GT and HT were switched. The two chimeras constructed are shown in Fig.1: ht-GT which consisted of the NH₂ terminal cytoplasmic tail of HT attached to the transmembrane, stem and catalytic domains of GT; and gt-HT which consisted of the NH₂ terminal 10 cytoplasmic tail of GT attached to the transmembrane, stem and catalytic domains of HT. The chimeric cDNAs were subcloned into the eukaryotic expression vector pcDNAI and used in transfection experiments.

The chimeric cDNAs encoding ht-GT and gt-HT were 15 initially evaluated for their ability to induce glycosyltransferase expression in COS cells, as measured by the surface expression of the appropriate sugar using lectins. Forty-eight hours after transfection COS cells were tested by immunofluorescence for their expression of 20 Gal- α (1,3)-Gal or H substance (Table 1 & Fig. 2). The staining with IB4 (lectin specific for Gal- α (1,3)-Gal) in cells expressing the chimera ht-GT (30% of cells stained positive) was indistinguishable from that of the normal GT staining (30%) (Table 1 & Fig. 2). Similarly the intense 25 cell surface fluorescence seen with UEAI staining (the lectin specific for H substance) in cells expressing gt-HT (50%) was similar to that seen in cells expressing wild-type pHt (50%) (Table 1 & Fig. 2). Furthermore, similar levels of mRNA expression of the glycosyltransferases GT 30 and HT and chimeric glycosyltransferases ht-GT and gt-HT were seen in Northern blots of total RNA isolated from transfected cells (Fig. 3). Thus both chimeric glycosyltransferases are efficiently expressed in COS cells and are functional indeed there was no detectable 35 difference between the chimeric and normal glycosyltransferases.

Glycosyltransferase activity in cells transfected with chimeric cDNAs encoding ht-GT and gt-HT

To determine whether switching the cytoplasmic tails of GT and HT altered the kinetics of enzyme function, 5 we compared the enzymatic activity of the chimeric glycosyltransferases with those of the normal enzymes in COS cells after transfection of the relevant cDNAs. By making extracts from transfected COS cells and performing GT or HT enzyme assays we found that N-acetyl lactosamine 10 was galactosylated by both GT and the chimeric enzyme ht-GT (Fig 4. panel A) over a the 1-5mM range of substrate concentrations. Lineweaver-Burk plots showed that both GT and ht-GT have a similar apparent Michealis-Menten constant of K_m 2. 6mM for N-acetyl lactosamine (Fig. 4. panel B). 15 Further HT, and the chimeric enzyme gt-HT were both able to fucosylate phenyl-B-D-galactoside over a range of concentrations (7. 5 - 25 mM) (Fig. 4 panel C) with a similar K_m of 2. 3mM (Fig. 4 panel D), in agreement with the reported K_m of 2. 4mM for HT (25). Therefore the 20 chimeric glycosyltransferases ht-GT and gt-HT are able to utilise N-acetyl lactosamine (ht-GT) and phenyl-B-D-galactoside (gt-HT) in the same way as the normal glycosyltransferases, thus switching the cytoplasmic domains of GT and HT does not alter the function of these 25 glycosyltransferases and if indeed the cytoplasmic tail is the localisation signal then both enzymes function as well with the GT signal as with the HT signal.

Switching cytoplasmic domains of GT and HT results in a reversal of the "dominance" of the glycosyltransferases

The cDNAs encoding the chimeric transferases or normal transferases were simultaneously co-transfected into COS cells and after 48h the cells were stained with either IB4 or UEA1 lectin to detect Gal- α (1,3)-Gal and H substance 35 respectively on the cell surface (Table 1 & Fig. 5). COS cells co-transfected with cDNAs for ht-GT + gt-HT (Fig 5 panel C) showed 30 % cells staining positive with IB4

(Table 1) but no staining on cells co-transfected with cDNAs for GT + HT (3%) (Fig. 5 panel A). Furthermore staining for H substance on the surface of ht-GT + gt-HT co-transflectants gave very few cells staining positive (5%) (Fig 5 panel D) compared to the staining seen in cells co-transfected with cDNAs for the normal transferases GT + HT (50%) (Fig. 5 panel B), ie. the expression of Gal- α (1,3)-Gal now dominates over that of H. Clearly, switching the cytoplasmic tails of GT and HT led to a complete reversal 10 in the glycosylation pattern seen with the normal transferases i.e. the cytoplasmic tail sequences dictate the pattern of carbohydrate expression observed.

That exchanging the cytoplasmic tails of GT and HT reverses the dominance of the carbohydrate epitopes 15 points to the glycosyltransferases being relocalized within the Golgi. To address this question, experiments were performed with cDNAs encoding glycosyltransferases with the same cytoplasmic tail: COS cells transfected with cDNAs encoding HT + ht-GT stained strongly with both UEAI (50%) 20 and IB4 (30%) (Table 1 & Fig. 5 panels E, F), the difference in staining reflecting differences in transfection efficiency of the cDNAs. Similarly cells transfected with cDNAs encoding GT + gt-HT also stained positive with UEAI (50%) and IB4 (30%) (Table 1 & Fig. 5 25 panel G, H). Thus, glycosyltransferases with the same cytoplasmic tail leads to equal cell surface expression of the carbohydrate epitopes, with no "dominance" of one glycosyltransferase over the other observed, and presumably the glycosyltransferases localised at the same site appear 30 to compete equally for the substrate.

In COS cells the levels of transcription of the cDNAs of chimeric and normal glycosyltransferases were essentially the same (Fig.3) and the immunofluorescence pattern of COS cells expressing the chimeric 35 glycosyltransferases ht-GT and gt-HT showed the typical staining pattern of the cell surface Gal- α (1,3)-Gal and H substance respectively (Table 1 & Fig. 2), the pattern

being indistinguishable from that of COS cells expressing normal GT and HT. Our studies showed that the Km of ht-GT for N-acetyl lactosamine was identical to the Km of GT for this substrate, similarly the Km of gt-HT for phenylBDgalactoside was approximately the same as the Km of HT for phenylBDgalactoside (Fig. 3). These findings indicate that the chimeric enzymes are functioning in a cytoplasmic tail-independent manner, such that the catalytic domains are entirely functional, and are in agreement with those of Henion et al (23), who showed that an NH₂ terminal truncated marmoset GT (including truncation of the cytoplasmic and transmembrane domains) maintained catalytic activity and confirmed that GT activity is indeed independent of the cytoplasmic domain sequence.

If the Golgi localisation signal for GT and HT is contained entirely within the cytoplasmic domains of the enzymes, then switching the cytoplasmic tails between the two transferases should allow a reversal of the order of glycosylation. Co-transfection of COS cells with cDNA encoding the chimeric glycosyltransferases ht-GT and gt-HT caused a reversal of staining observed with the wild type glycosyltransferases (Fig. 5), demonstrating that the order of glycosylation has been altered by exchanging the cytoplasmic tails. Furthermore, co-transfection with cDNA encoding glycosyltransferases with the same cytoplasmic tails (i. e. HT + ht-GT and GT + gt-HT) gave rise to equal expression of both Gal- α (1,3)-Gal and H substance (Fig.5). The results imply that the cytoplasmic tails of GT and HT are sufficient for the localisation and retention of these two enzymes within the Golgi.

To date only twenty or so of at least one hundred predicted glycosyltransferases have been cloned and few of these have been studied with respect to their Golgi localisation and retention signals (34). Studies using the elongation transferase N-acetylglucosaminyltransferase I (33-37), the terminal transferases α (2,6)sialyltransferase (24-26) and β (1,4)galactosyltransferase (38-40) point to

residues contained within the cytoplasmic tail, transmembrane and flanking stem regions as being critical for Golgi localisation and retention. There are several examples of localisation signals existing within 5 cytoplasmic tail domains of proteins including the KDEL and KKXX motifs in proteins resident within the endoplasmic reticulum (41,42) the latter motif also having been identified in the cis Golgi resident protein ERGIC-53 (43) and a di-leucine containing peptide motif in the mannose-6- 10 phosphate receptor which directs the receptor from the trans-Golgi network to endosomes (44). These motifs are not present within the cytoplasmic tail sequences of HT or GT or in any other reported glycosyltransferase. To date a 15 localisation signal in Golgi resident glycosyltransferases has not been identified and while there is consensus that transmembrane domains are important in Golgi localisation, it is apparent that this domain is not essential for the localisation of all glycosyltransferases, as shown by the study of Munro (45) where replacement of the transmembrane 20 domain of $\alpha(2,6)$ sialyltransferase in a hybrid protein with a poly-leucine tract resulted in normal Golgi retention. Dahdal and Colley (46) also showed that sequences in the transmembrane domain were not essential to Golgi retention. This study is the first to identify sequence 25 requirements for the localisation of $\alpha(1,2)$ fucosyltransferase and $\alpha(1,3)$ galactosyltransferase within the Golgi. It is anticipated that other glycosyltransferases will have similar localisation mechanisms.

30

Example 2 Use of secretor in construction of a chimeric enzyme

A construct is made using PCR and subcloning as described in Example 1, such that amino acids #1 to #6 of 35 the pig $\alpha(1,3)$ -galactosyltransferase (MNVKGR) replace amino acids #1 to 5 of the pig secretor (Fig 6). Constructs are tested as described in Example 1.

Example 3 Use of pig H transferase in construction of a chimeric enzyme

5 A construct is made using PCR and subcloning as described in Example 1, such that amino acids #1 to #6 of the pig $\alpha(1,3)$ -galactosyltransferase (MNVKGR) replace amino acids #1 to 8 of the pig H transferase (Fig 7). Constructs are tested as described in Example 1.

10 Example 4. Generation of pig endothelial cells expressing chimeric $\alpha(1,2)$ fucosyltransferase

15 The pig endothelial cell line PIEC expressing the chimeric $\alpha 1,2$ fucosyltransferase was produced by lipofectamine transfection of pgtHT plasmid DNA (20 μ g) and pSV2NEO (2 μ g) and selecting for stable integration by growing the transfected PIEC in media containing G418 (500 μ g/ml; Gibco-BRL, Gaithersburg, MD). Fourteen independant clones were examined for cell surface expression of H substance by staining with UEA-1 lectin. >95% of cells of 20 each of these clones were found to be positive. Fig. 8 shows a typical FACS profile obtained for these clones.

Example 5 Production of transgenic mice expressing chimeric $\alpha(1,2)$ fucosyltransferase

25 A NruI/NotI DNA fragment, encoding the full length chimeric $\alpha 1,2$ fucosyltransferase, was generated utilising the Polymerase Chain Reaction and the phHT plasmid using the primers:

5' primer homologous to the 5'UTR:

30 5'-TTCGCGAATGAATGTCAAAGGAAGACTCTG, in which the underlined sequence contains a unique NruI site;

3' primer homologous to the 3'UTR:

5'-GGCGGCCGCTCAGATGTTATTCTAACCAAAT

the underlined sequence contains a NotI site

35 The DNA was purified on gels, electroeluted and subcloned into a NruI/NotI cut genomic H-2Kb containing vector resulting in the plasmid clone (pH-2Kb-gtHT)

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encoding the chimeric $\alpha(1,2)$ -fucosyltransferase gene directionally cloned into exon 1 of the murine H-2Kb gene, resulting in a transcript that commences at the H-2Kb transcriptional start site, continuing through the gtHT 5 cDNA insert. The construct was engineered such that translation would begin at the initiation codon (ATG) of the hHT cDNA and terminate at the in-phase stop codon (TGA).

DNA was prepared for microinjection by digesting 10 pH-2Kb-hHT with *Xba*I and purification of the H-2Kb-hHT DNA from vector by electrophoretic separation in agarose gels, followed by extraction with chloroform, and precipitation 15 in ethanol to decontaminate the DNA. Injections were performed into the pronuclear membrane of (C57BL/6xSJL)F1 zygotes at concentrations between 2-5ng/ml, and the zygotes transferred to pseudopregnant (C57BL/6xSJL)F1 females.

The presence of the transgene in the live 20 offspring was detected by dot blotting. 5mg of genomic DNA was transferred to nylon filters and hybridized with the insert from gtHT, using a final wash at 68°C in 0.1xSSC/1% SDS. Fig. 9 shows the results of testing 12 live 25 offspring, with two mice having the transgenic construct integrated into the genome. Expression of transgenic protein is examined by estimating the amount of UEA1 lectin (specific for H substance) or anti-H mAb required to haemagglutinate red blood cells from transgenic mice. Hemagglutination in this assay demonstrates transgene expression.

It will be apparent to the person skilled in the 30 art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this 35 specification.

References cited herein are listed on the following pages, and are incorporated herein by this

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reference.

TABLE 1

EXPRESSION OF GAL- α (1,3)GAL AND H SUBSTANCE BY COS CELLS
TRANSFECTED WITH cDNAs ENCODING NORMAL AND CHIMERIC

5 GLYCOSYLTRANSFERASES

COS cells transfected with cDNA encoding:	%IB4 positive cells	%UEAI positive cells
GT	30	0
HT	0	50
ht-GT	30	0
gt-HT	3	50
GT+HT	3	50
ht-GT+gt-HT	33	5
GT+gt-HT	30	30
GT+ht-GT	30	0
HT+ht-GT	30	30
HT+gt-HT	0	50
Mock	0	0

Transfected COS cells were stained with FITC-labelled IB4 (lectin specific for Gal- α (1,3)Gal or UEAI (lectin specific for H substance) and positive staining cells were visualized and counted by fluorescence microscopy. Results are from at least three replicates.

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CLAIMS

1. A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby when said nucleic acid is expressed in a cell said chimeric enzyme is located in an area of the cell where it is able to compete for substrate with a second glycosyltransferase, resulting in reduced levels of a product from said second glycosyltransferase.
2. A nucleic acid according to claim 1, wherein said localisation signal localises said catalytic domain thereby to enable the catalytic domain to compete with said second glycosyltransferase for a substrate.
3. A nucleic acid according to claim 1 or claim 2, wherein the localisation signal is derived from a glycosyltransferase which produces glycosylation patterns which are recognised as foreign by a transplant recipient.
4. A nucleic acid according to any one of claims 1 to 3, wherein the localisation signal comprises the amino terminus of the second glycosyltransferase.
5. A nucleic acid according to any one of claims 1 to 4, wherein the localisation signal is derived from $\alpha(1,3)$ -galactosyltransferase.
6. A nucleic acid according to any one of claims 1 to 5, wherein the first glycosyltransferase is selected from the group consisting of H-transferase, secretor sialyltransferase, a galactosyl sulphating enzyme or a phosphorylating enzyme.
7. A nucleic acid according to any one of claims 1 to 6, wherein the catalytic domain and the localisation signal each originates from a mammal selected from the group consisting of human, primates, ungulates, dogs, mice, rats and rabbits.
8. A nucleic acid according to any one of claims 1 to 7, wherein the localisation signal is derived from the

same species as the cell which the nucleic acid is intended to transform.

9. A nucleic acid according to any one of claims 1 to 8, comprising a sequence encoding the catalytic domain of H transferase and a nucleic acid sequence encoding a localisation signal from Gal transferase.

10. A nucleic acid according to claim 9, wherein the catalytic domain and the localisation signal are derived from pigs.

11. A nucleic acid according to any one of claims 1 to 10, which encodes gtHT as defined herein.

12. A vehicle comprising a nucleic acid according to any one of claims 1 to 11.

13. vehicle according to claim 12, selected from the group consisting of an expression vector, plasmid and phage.

14. A vehicle according to claim 12 or claim 13, which enables said nucleic acid to be expressed in prokaryotes or in eukaryotes.

15. An isolated nucleic acid molecule encoding a localisation signal of a glycosyltransferase.

16. An isolated nucleic acid molecule according to claim 15, wherein the signal encoded comprises an amino terminus of gal-transferase.

17. A method of producing a nucleic acid according to any one of claims 1 to 11, comprising the step of operably linking a nucleic acid sequence encoding a catalytic domain from a first glycosyltransferase to a nucleic acid sequence encoding a localisation signal of a second glycosyltransferase.

18. A method of reducing the level of a carbohydrate exhibited on the surface of a cell, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the

cell where it is able to compete for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

5 19. A method of producing a cell from a donor species which is immunologically acceptable to a recipient species by reducing levels of carbohydrate on said cell which cause it to be recognised as non-self by the recipient, said method comprising causing a nucleic acid to be expressed in
10 said cell wherein said nucleic acid encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localisation signal of a second glycosyltransferase, whereby said chimeric enzyme is located in an area of the cell where it is able to compete
15 for substrate with said second glycosyltransferase, and wherein said second glycosyltransferase is capable of producing said carbohydrate.

20. A cell produced by a method according to claim 19.

20 21. An organ comprising a cell according to claim 20.

22. A non-human transgenic animal, organ or cell comprising the nucleic acid according to any one of claims 1 to 11.

25 23. An expression unit which expresses a nucleic acid according to any one of claims 1 to 11, resulting in a cell which is immunologically acceptable to an animal having reduced levels of a carbohydrate on its surface, which carbohydrate is recognised as non-self by said species.

24. An expression unit according to claim 23,
30 selected from the group consisting of a retroviral-packaging cassette, retroviral construct or retroviral producer cell.

25 25. A method of producing an expression unit according to claim 23 or claim 24, said unit having reduced levels of a carbohydrate on its surface wherein the carbohydrate is recognised as non-self by a species, comprising transforming/transfected a retroviral packaging

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cell or a retroviral producer cell with the nucleic acid of the invention under conditions such that the chimeric enzyme is produced.

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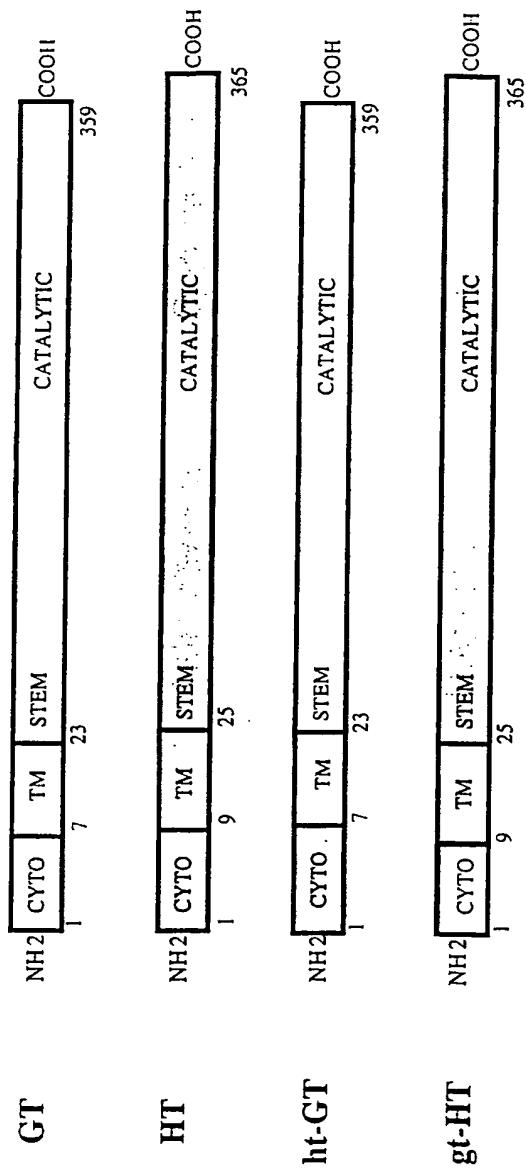


FIGURE 1

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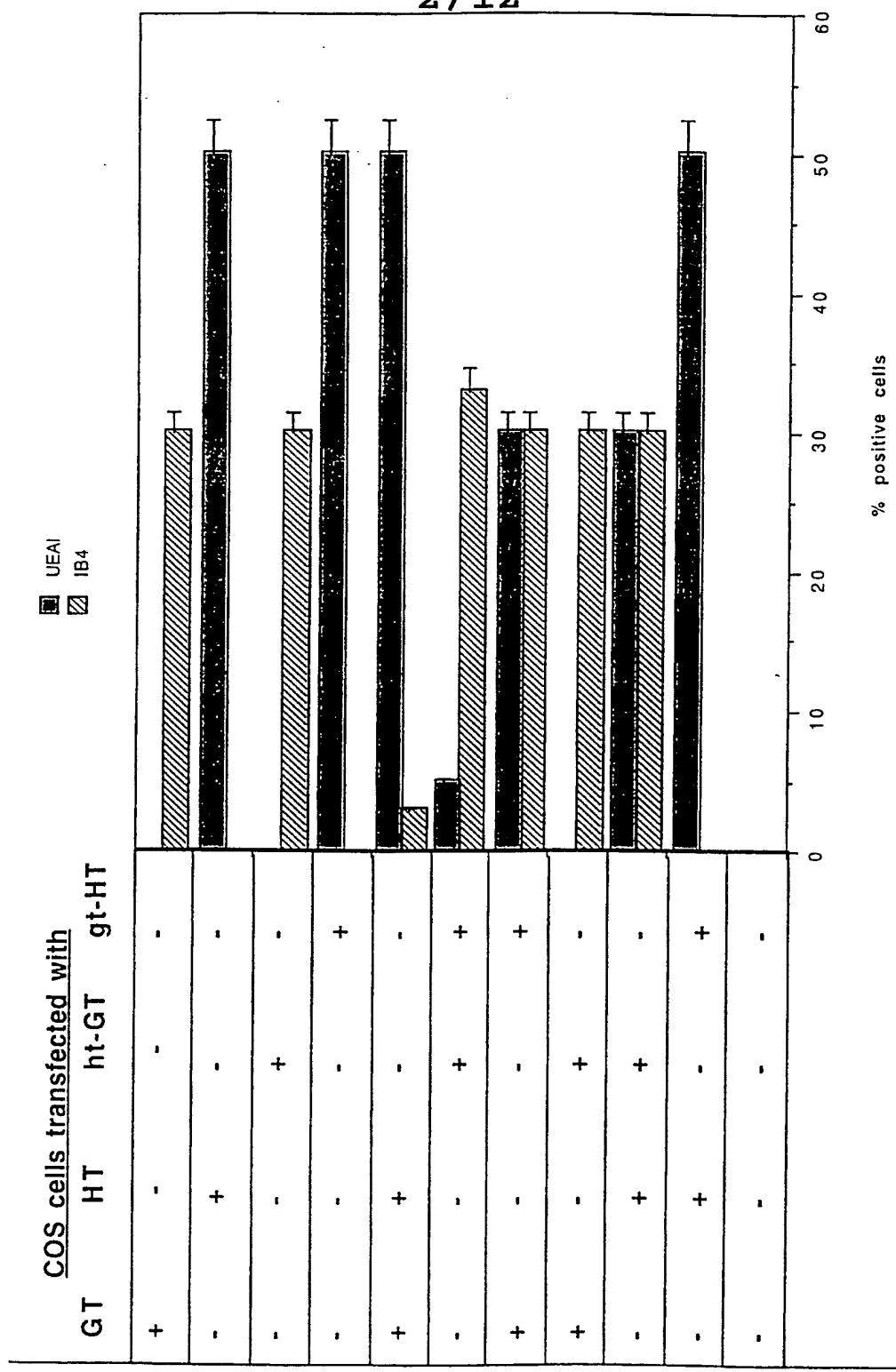


FIGURE 2

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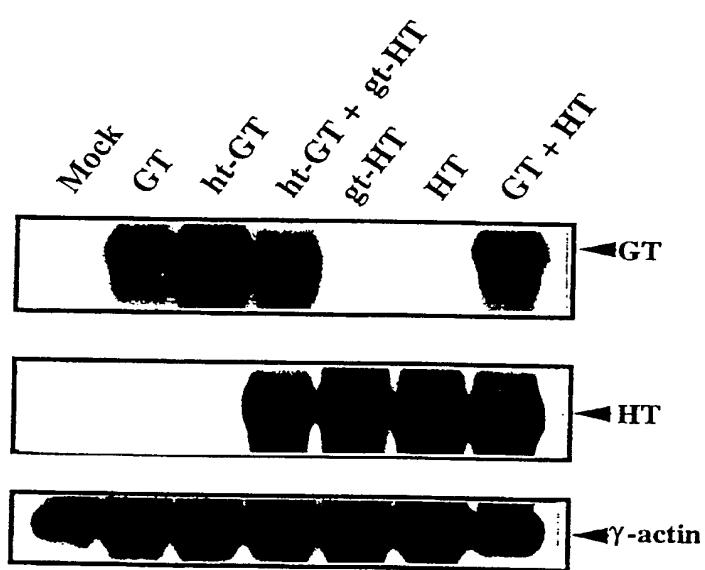


FIGURE 3

SUBSTITUTE SHEET (Rule 26)

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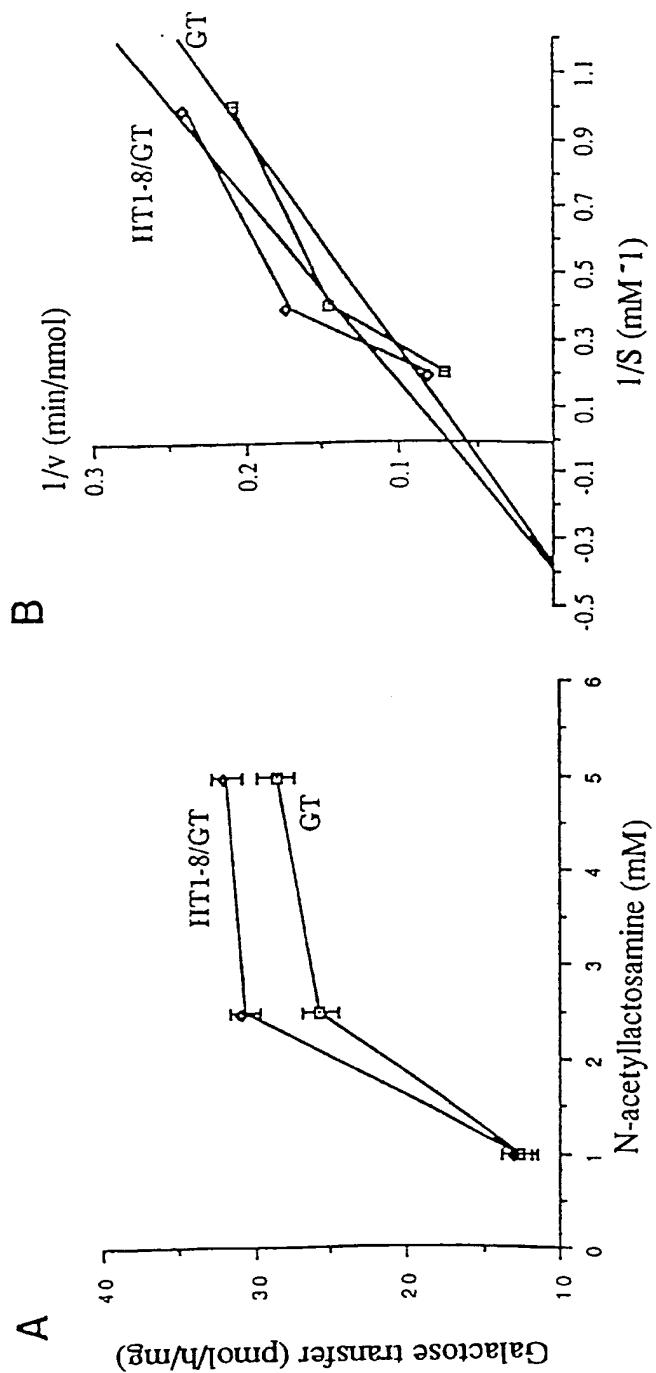


FIGURE 4

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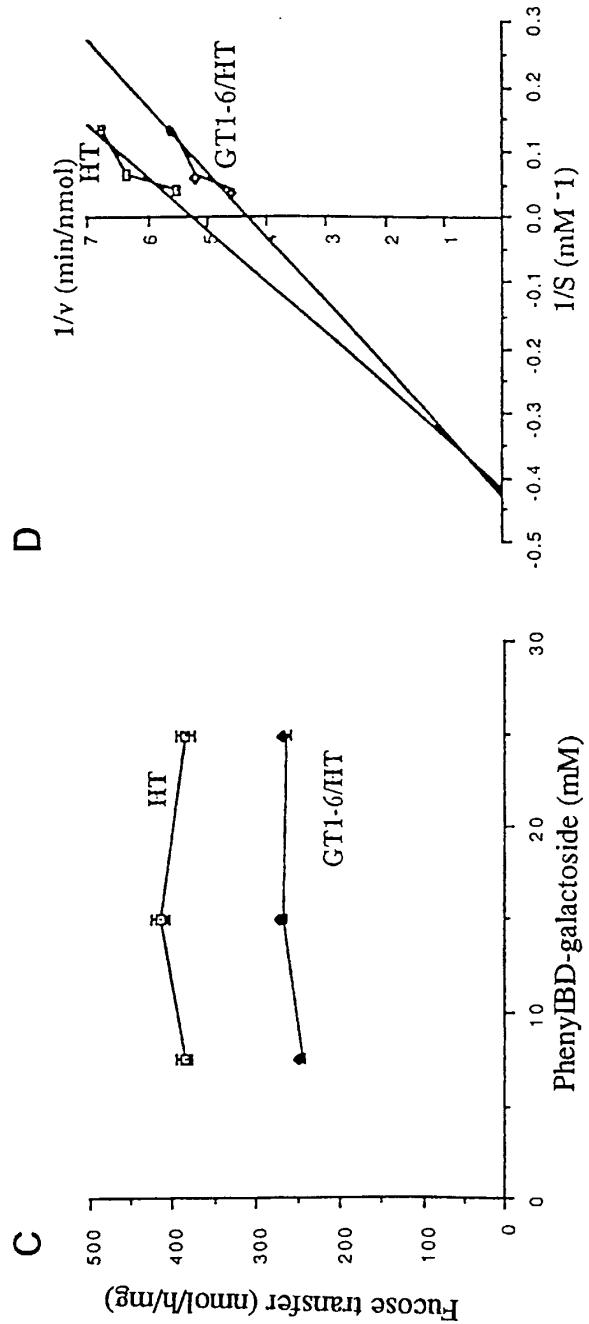


FIGURE 4 Continued

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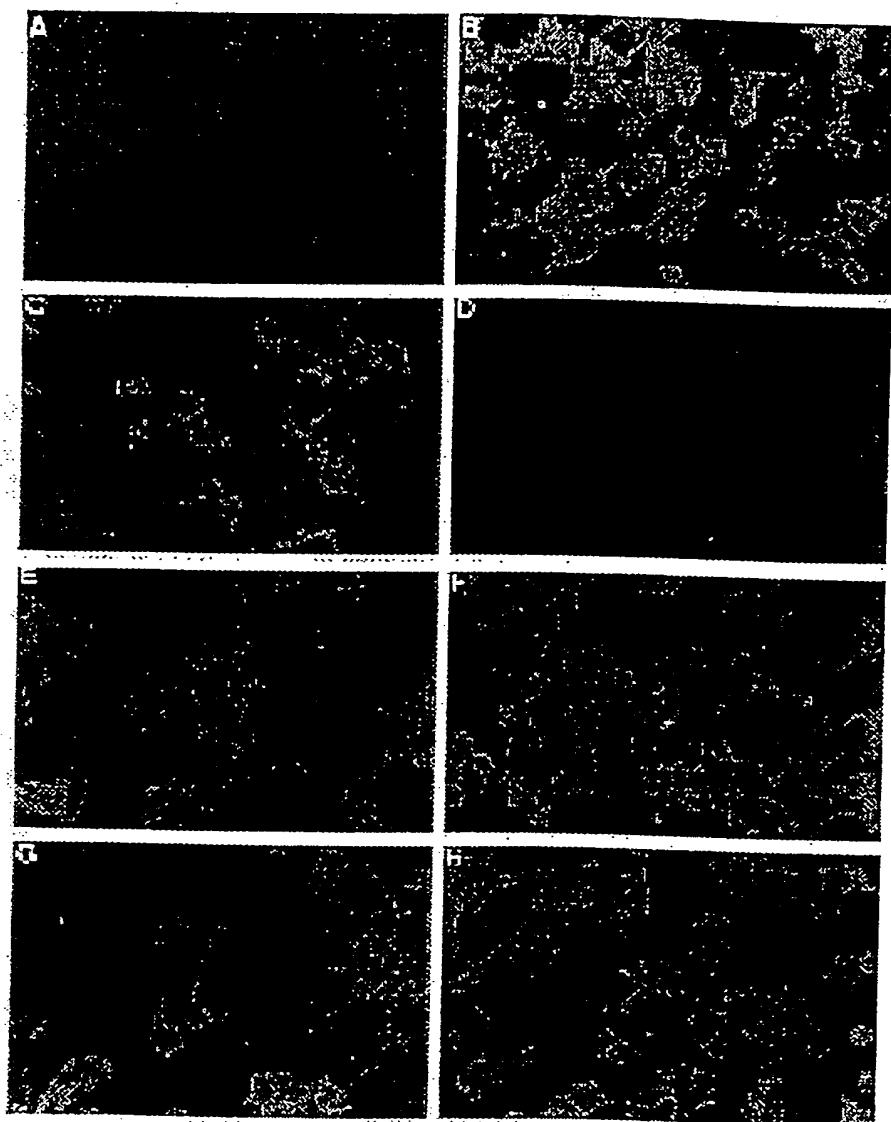


FIGURE 5

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PORCINE SECRETOR SEQUENCE

CT	ACA	GCC	ATG	CTC	AGC	ATG	CAG	GCA	TCC	TTC	TTC	CCC	ACG	GGT	CCC	TTC	ATC	CTC	59	
F	V	F	T	A	S	T	I	F	H	L	Q	Q	R	N	V	K	I	Q	P	37
TTT	GTC	TTC	ACG	GCT	TCC	ACC	ATA	TTT	CAC	CTT	CAG	CAG	AGG	ATG	GTG	AAG	ATT	CAA	CCC	119
T	W	E	L	Q	M	V	T	Q	V	T	T	E	S	P	S	S	P	Q	L	57
ACG	TGG	GAG	TTA	CAG	ATG	GTG	ACG	CAG	GTG	ACC	ACA	GAG	AGC	CCC	TCG	AGC	CCC	CAG	CTG	179

PORCINE SECRETOR SEQUENCE

K	G	M	W	T	I	N	A	I	G	R	L	G	N	Q	M	G	E	Y	A	77
AAG	GGC	ATG	TGG	ACG	ATC	AAT	GCC	ATC	GGC	CGC	CTG	GGG	AAC	CAG	ATG	GGG	GAG	TAC	GCC	239
T	L	Y	A	L	A	R	M	N	G	R	P	A	F	I	P	P	E	M	H	97
ACC	CTG	TAC	GCG	CTG	GCC	AGG	ATG	AAC	GGG	CGG	CCG	GCC	TTC	ATC	CCG	CCC	GAG	ATG	CAC	299
S	T	L	A	P	I	F	R	I	T	L	P	V	L	H	A	S	T	A	R	117
AGC	ACG	CTG	GCC	CCC	ATC	TTC	AGG	ATC	ACC	CTC	CCG	GTC	CAC	GCC	AGC	ACG	GCC	CGC	359	
R	I	P	W	Q	N	Y	H	L	N	D	W	M	E	E	R	Y	R	H	I	137
AGG	ATC	CCC	TGG	CAG	AAC	TAC	CAC	CTG	AAC	GAC	TGG	ATG	GAG	CGG	TAC	CGC	CAC	ATC	419	
P	G	E	Y	V	R	L	T	G	Y	P	C	S	W	T	F	Y	H	H	L	157

FIGURE 6

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CCG	GGG	GAG	TAC	GTG	CGC	CTC	ACG	GGC	TAC	CCC	TGC	TCC	TGG	ACC	TTC	TAC	CAC	CAC	CTG	479
R	T	E	I	L	R	E	F	T	L	H	N	H	V	R	E	E	A	Q	D	177
CGC	ACC	GAG	ATC	CTC	CGG	GAG	TTC	ACC	CTG	CAT	AAC	CAC	CTG	CGC	GAG	GAC	CAG	GAT	539	
F	L	R	G	L	R	V	N	G	S	R	P	S	T	Y	V	G	V	H	V	197
TTC	CTG	CGG	GGT	CTG	CGG	GTG	AAC	GGG	AGC	CGA	CCG	AGT	ACC	TAC	GTG	GGG	GTG	CAC	GTG	599
R	R	G	D	Y	V	H	V	M	P	N	V	W	K	G	V	V	A	D	R	217
CGC	CGG	GAC	TAC	GTG	CAC	GTG	ATG	CCC	AAC	GTG	TGG	AAG	GAC	GTG	GTG	GCC	GAC	CGG	659	
R	Y	L	E	Q	A	L	D	W	F	R	A	R	Y	R	S	P	V	F	V	237
CGG	TAC	CTG	GAG	CAG	GCC	CTG	GAC	TGG	TTC	CGG	GCT	CGC	TAC	CGC	TCC	CCC	GTC	TTT	GTG	719
V	S	S	N	G	M	A	W	C	R	E	N	I	N	A	S	R	G	D	V	257
GTC	TCC	AGC	AAC	GGC	ATG	GCC	TGG	TGT	CGG	GAA	AAC	ATC	AAT	GCC	TCG	CGC	GAC	GAT	GTG	779
V	F	A	G	N	G	I	E	G	S	P	A	K	D	F	A	L	L	T	Q	277
TGT	TTT	GCC	GGC	AAT	GGC	ATC	GAG	GGC	TCC	CCC	GCC	AAA	GAC	TTC	GGC	CTG	CTC	ACG	CAG	839
C	N	H	T	V	M	T	I	G	T	F	G	I	W	A	A	Y	L	A	G	297
TGT	AAC	CAC	ACT	GTC	ATG	ACC	ATT	GGC	ACG	TTC	GGG	ATC	TGG	GCC	GCC	TAC	CTT	GCT	GGT	899
G	E	T	I	Y	L	A	N	Y	T	L	P	D	S	P	F	L	K	L	F	317
GGA	GAG	ACC	ATC	TAC	CTG	GCC	AAT	TAC	ACG	CTC	CCG	GAC	TCT	CCC	TTC	CTC	AAA	CTC	TTT	959

FIGURE 6 (cont.)**SUBSTITUTE SHEET (RULE 26)**

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K	P	E	A	A	F	L	P	E	W	I	G	I	E	A	D	L	S	P	L	337
AAG	CCC	GAG	GCA	GCC	TTC	CTG	CCC	GAG	TGG	ATT	GGG	ATC	GAG	GCA	GAC	CTG	TCC	CCA	CTC	1019
L	K	H	*																	340
CTT	AAG	CAC	TGA	TGT	CGG	CTG	TCC													1043

FIGURE 6 (cont.)**SUBSTITUTE SHEET (RULE 26)**

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PIG H TRANSFERASE

M W V P S R R H L C L T F L - L V C V L A	20
ATGTGGGTCCCCAGCCGGCGCCACCTCTGCTCTGACCTTCCCTGCTAGTCTGTGTTTAGCA	60
A I F F L N V Y Q D L F Y S G L D L L A	40
GCAATTCTTCCTGAACGTCTATCAAGACCTCTTTACAGTGGCTTAGACCTGCTGGCC	120
L C P D H N V V S S P V A I F C L A G T	60
CTGTGTCCAGACCATAACGTGGTATCATCTCCCGTGGCCATATTCTGCCTGGCGGGACG	130
P V H P N A S D S C P K H P A S F S G T	80
CCGGTACACCCCCAACGCCCTCCGATTCTGTCCCAGCATTCTGCCTCCTTTCCGGGACCC	240
W T I Y P D G R F G N Q M G Q Y A T L L	100
TGGACTATTTACCCGGATGGCCGGTTGGGAACCAGATGGGACAGTATGCCACGCTGCTG	300
A L A Q L N G R Q A F I Q P A M H A V L	120
CCCCCTGGCGCAGCTAACGGCCGCCAGGCCCTCATCCAGCCTGCCATGCACGCCGTCTG	360
A P V F R I T L P V L A P E V D R H A P	140
CCCCCGTGTCCGCATCACGCTGCTGTCCCTGGCCCGAGGTAGACAGGCACGCTCCT	420
W R E L E L H D W M S E D Y A H L K E P	160
TGGCGGGAGCTGGAGCTTCACGACTGGATGTCCGAGGATTATGCCCACTAAAGGAGCCC	480
W L K L T G F P C S W T F F H H L R E Q	180
TGGCTGAAGCTCACCGGTTCCCTGCTCCCTGGACCTTCTCCACCACTCCGGGAGCAG	540
I P S E F T L H D H L R Q E A Q G V L S	200
ATCCGCAGCGAGTTCACCCCTGCACGACCACCTCGGCAAGAGGCCAGGGGTACTGAGT	600
Q F R L P R T G D R P S T F V G V H V R	220
CAGTTCCGTCTACCCCGCACAGGGgACCGCCCCAGCACCTTCGTGGGGTCCACGTGCGC	660
R G D Y L R V M P K R W K G V V G D G A	240
CGCGGGGACTATCTGCGTGTGATGCCAACCGCTGGAAAGGGGGTGGTGGTACGGCGCT	720
Y L Q Q A M D W F R A R Y E A P V F V V	260
TACCTCCAGCAGGCTATGGACTGGTTCCGGGCCGATAACGAAGCCCCCTTTGTGGTC	780
T S N G M E W C R K N I D T S R G D V I	280
ACCAGCAACGGCATGGAGTGGTGGCCGGAAAGAACATCGACACCTCCGGGGGACGTGATC	840
F A G D G R E A A P A R D F A L L V Q C	300
TTTGCCTGGCGATGGCGGAGGGCGCGCCAGGGACTTGCCTGGTGCAGTGC	900
N H T I M T I G T F G F W A A A Y L A G G	320
AACCACACCATCATGACCATGGCACCTTGGCTTCTGGCCGCTACCTGGCTGGTGG	960
D T I Y L A N F T L P T S S F L K I F K	340
GATACCATCTACTTGCTACTTCACCCCTGCCACTTCCAGCTTCTGAAGATCTTAAA	1020
P E A A F L P E W V G I N A D L S P L Q	360
CcCGAGGCTGCCTTCTGCCAGTGGTGGCATTAATGCAGACTTGTCTCCACTCCAG	1080
M L A G P *	365
ATGTTGGCTGGGGCTTGA	1098

FIGURE 7

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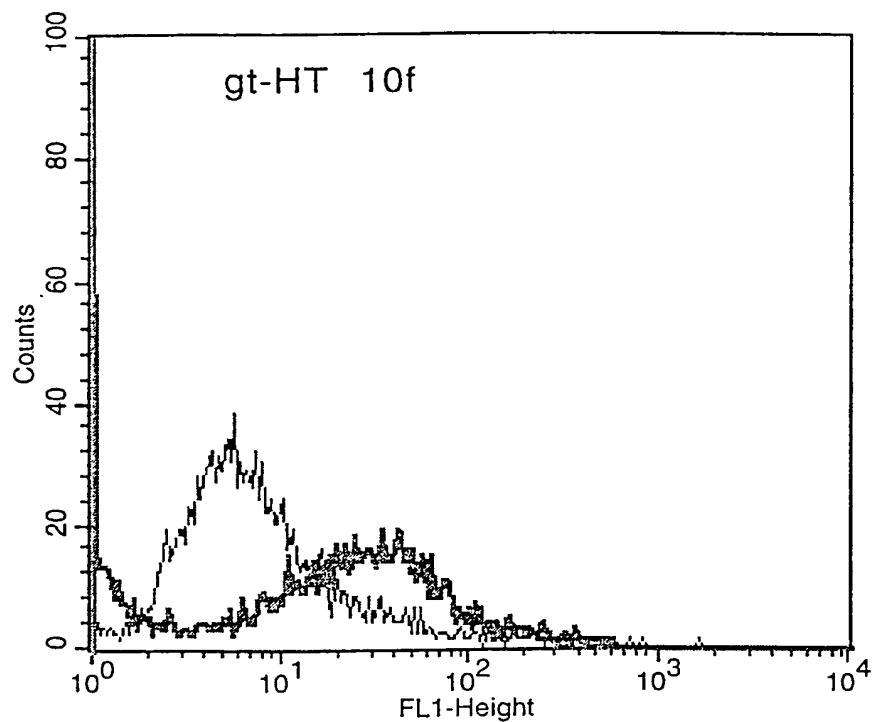


FIGURE 8
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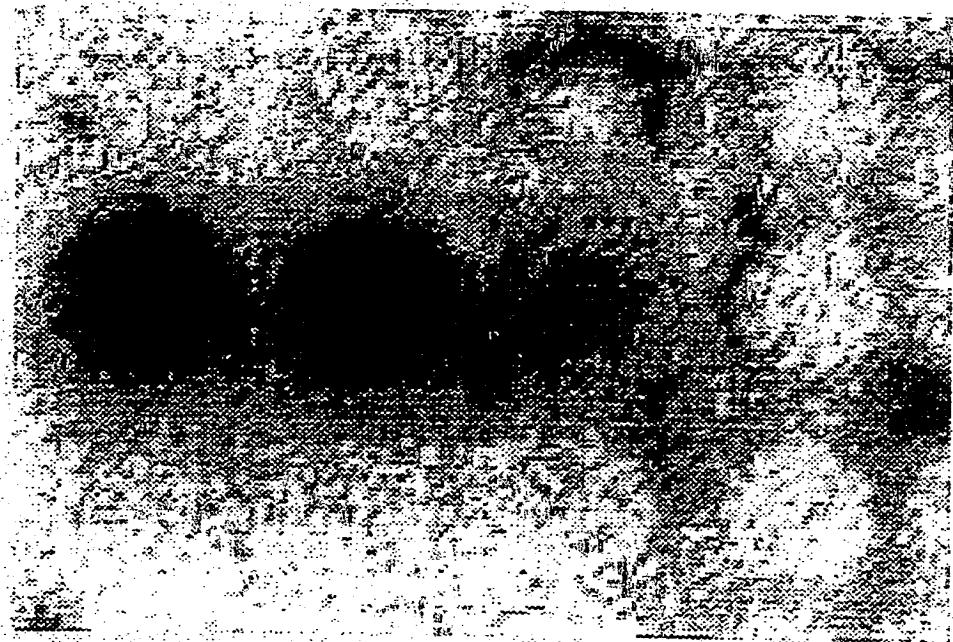


FIGURE 9
SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00492

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl⁶: C12N 15/11, 15/62, 5/16, 15/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
WPAT C12N 15/62 AND 15/54 and Chemical Abstracts see below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
STN:Medline see below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT C12N 15/62 AND (ENZYME# OR TRANSFERASE#) C12N 15/54, AND (CHIMER:OR CHIMAER:OR FUSION# OR MUTANT# OR HYBRID#) for STN and Chem Abs see attached

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Nature Medicine 1 (12) pages 1261-67 (1995) Sandrin, M. S. et al "Enzymatic remodelling of the carbohydrate surface of a xenogenic cell substantially reduces human antibody binding and complement-mediated cytosis"	

Further documents are listed in the continuation of Box C

See patent family annex

* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means		document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	"&"	

Date of the actual completion of the international search	Date of mailing of the international search report 22 OCT 1997
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer JIM CHAN Telephone No.: (02) 6283 2340

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00492

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 94 12646 (CIBA GEIGY AC) 9 June 1994	
A	J. Biol. Chem. 269 (16) pages 12049-59 (1994) Burke, J. et al "Medial-Golgi Retention of N-Acetylglucosaminyl transferase I".	
PX	J. Biol. Chem. (1996) 271 (51) pages 33105-33109. Osman, N. et al "Switching Amino-terminal cytoplasmic Domains of α (1, 2) Fucosyltransferase and α (1, 3) Galactosyltransferase Alters the Expression of H Substance and Gal α (1, 3) Gal.	

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00492

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00492

Box

Continuation of **FIELDS SEARCHED BOX B** Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN MEDLINE (glycosyltransferase/CT OR galactosyltransferase/CT OR sialyltransferase/CT OR fucosyltransferase/CT OR glucosyltransferase/CT) AND (chimera or chimeric or fusion or hybrid)

CHEM ABS (glycosyltransferase/IT OR galactosyltransferase/IT OR sialyltransferase/IT OR fucosyltransferase/IT OR glucosyltransferase/IT) AND (chimera or chimeric or fusion or hybrid) AND 7-5/ccError! Bookmark not defined.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 97/00492

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	9412646	AU	940622
		CA	940609
		EP	970813
		FI	950526
		MX	940831
		NO	950523
		US	970624
		WO	940609

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